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硕士研究生学位论文

基于微球技术的流式细胞术检测

下呼吸道感染病原菌方法的建立

Microsphere-Based Flow Cytometry for the
Microbiological Diagnosis of Lower Respiratory Tract
Infection

作者姓名 黄松洁

指导教师姓名: 欧阳高亮 杨天赐 教授

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摘 要

传统的流式细胞术所能获得的参数主要限于细胞、细菌或颗粒的大小、形状及其比率，难以对生物医学上有重要意义的核酸、蛋白质进行检测分析。但随着化学材料科学的发展，各种人工微球应运而生，以微球为基础，流式细胞检测技术得到迅速的发展，其应用领域也得到极大的拓展。

本实验拟以聚苯乙烯微球为载体，通过共价结合方式结合核酸捕获探针，用来特异性识别并捕获待测核酸序列，再加上荧光素标记的核酸检测探针，三者在同一液相体系中杂交，然后应用流式细胞技术进行检测分析。另外，我们利用不同大小的微球和标记不同荧光素的探针进行组合编码，从而实现了在同一检测中对不同核酸靶序列的多重检测。

我们将该多重检测方法应用于下呼吸道感染常见病原菌的检测。我们通过多重PCR反应扩增了大肠埃希菌、肺炎克雷伯菌、铜绿假单胞菌、肺炎链球菌等下呼吸道感染常见病原菌的靶基因序列，并将靶基因序列与不同大小的微球及标记不同荧光素的探针进行液相杂交，最后应用流式细胞术进行检测分析。

本实验灵敏度主要取决于多重PCR的灵敏度，实验中多重PCR灵敏度达500copies/ml，可以满足临床需求。多重PCR实验经优化后，各菌株DNA的扩增效率较一致，经流式细胞仪检测后，阳性微球百分率分别为：大肠埃希菌18.7%，肺炎克雷伯菌17.8%，铜绿假单胞菌19.2%，肺炎链球菌20.1%。在液相杂交中未发现明显的非特异性杂交。液相杂交及流式细胞术检测重复性良好，批内CV在3.46%~5.00%之间。对40例临床细菌培养分离菌株的检测阳性率为100%。因此，本实验建立的基于微球技术的流式细胞术检测下呼吸道感染病原菌的方法灵敏度高、重复性良好、特异性强。但要在临床推广应用，尚需进一步优化实验、控制好背景荧光的稳定性及对实验数据进行更科学的分析处理。

关键词：流式细胞术；微球；下呼吸道感染

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Abstract

Traditionally the flow cytometry could be used to analyze the size, shape and proportion of cells, bacteria or particle, but could not be used to detect and analyze the nucleic acids or proteins. But with the development of chemical materials science, all kinds of artificial microspheres emerged. Basing on microspheres, flow cytometry has been developed rapidly, and its applications have also been greatly extended.

Carboxy functionalized polystyrene microsphere is an important carrier in our experiment. The external carboxy group can be activated to couple with the probes. The microspheres coupled with nucleic acid probe capture the analytes which then bind with fluorescent-labeled nucleic acid probes. Through the detection and analysis of the microsphere fluorescence intensity and the microsphere size by the flow cytometry, the analytes can be identified significantly.

The multiple-detection method was applied for the detection of lower respiratory tract infection pathogenic bacteria, we amplified the target gene sequences of *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* and *Streptococcus pneumoniae* by multiplex PCR, then the target gene sequences and the microspheres coupled with probes and the fluorescent-labeled probes were hybridized in liquid-phase, finally the target gene sequences were detected and analyzed by flow cytometry.

The experimental sensitivity is mainly depends on the sensitivity of multiplex PCR, which sensitivity is up to 500 copies/ml. The amplification efficiency of 4 strains DNA is not obviously different to each other, after being detected by flow cytometry, positive rate of microspheres is as follows: *Escherichia coli* 18.7%, *Klebsiella pneumoniae* 17.8%, *Pseudomonas aeruginosa* 19.2%, *Streptococcus pneumoniae* 20.1%. There is no significant non-specific hybridization. The reproducibility of Liquid-phase hybridization and flow cytometric detection is acceptable, coefficient variation of five repeat detections was 3.46%~5.00%. 40 separated strains were identified successfully, positive rate is 100%. Microsphere-based flow cytometry for the microbiological

diagnosis of lower respiratory tract infection is developed. This experiment can exactly identify 1 to 4 target pathogenic bacteria in the same reaction with better sensitivity, specificity and reproducibility. but there are also some important problems suggested to be solved which include optimization of experiment, stability of background fluorescence and scientific analysis of numerical data.

Keywords: flow cytometry; microsphere; lower respiratory tract infection

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